DEVELOPMENTS OF TOXICOLOGICAL AND PHYSIOLOGICAL STUDIES ON FORMALDEHYDE

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ABSTRACT
This paper is a review on recent developments of the toxicological and physiological studies on formaldehyde. Hotspots involved in this topic include: (1) Formaldehyde-induced gene expression; (2) Formaldehyde-induced asthma; (3) Airway neurogenic inflammation (4) formaldehyde working as signaling molecule in body; (5) Epigenesis: methylation of DNA and proteins; (6) DNA-protein crosslink; (7) Lysine-DPC repair enzyme; (8) Formaldehyde-induced Apoptosis.

INDEX TERMS
Formaldehyde, Toxicological study, Physiological study

INTRODUCTION

On 15 June 2004 International Agency for Research on Cancer of WHO issued an important press release "IARC Classifies Formaldehyde as Carcinogenic to Humans", in which the first paragraph said: "Twenty-six scientists from 10 countries evaluated the available evidence on the carcinogenicity of formaldehyde, …the working group …concluded that formaldehyde is ***carcinogenic to humans***. Previous evaluations, based on the smaller number of studies available at that time, had concluded that formaldehyde was ***probably carcinogenic to humans***, but new information from studies of persons exposed to formaldehyde has increased the overall weight of the evidence." (WHO, 2004) No suspense any more now has about the carcinogenicity of formaldehyde. This is a great progress for the risk assessment of formaldehyde, which has been in use of a biologically based dose-response (BBDR) model since 1996 (Haber LT et al, 2001), over 30-year effort the conclusion finally comes out.

Whether do the studies on formaldehyde go to end or over? No, new hotspots about the biological effects of formaldehyde have emerged in the field of biomedical and environmental science. It is also noticed that recent developments in molecular biology, including transgenic animals, microarrays, and characterization of genetic polymorphisms, have potential for improving risk assessments, although further methods development is needed. In this review the authors have collected some of the new hotspots and present them to conference attendee.

FORMALDEHYDE-INDUCED GENE EXPRESSION

Two or three very new academic papers have been published on this topic. For example:

1 Hester SD et al from USA (2003) published their paper "formaldehyde-induced gene expression in F344 rat nasal respiratory epithelium" on Toxicology 187:13-24. Two groups of male F344 rats received either 40 µl of distilled water or FA (400 mM) instilled into each nostril. 24 hours following treatment, nasal epithelium cells was recovered from which total RNA was used to generate cDNA probes. Significance analysis of microarrays (SAM) hybridization data using Clontech^{TM} Rat Atlas 1.2 arrays revealed that 24 of the 1185 genes queried were significantly upregulated and 22 genes were significantly downregulated. Results for ten of the differentially expressed genes were confirmed by quantitative real time RT PCR. The identified genes with FA-induced change in expression belong to the functional gene categories xenobiotic metabolism, cell cycle, apoptosis, and DNA repair.

2 Zhiqian Tong et al (2005) submitted the paper "up-regulation of the mRNA-transcripts of VR1 and NGF induced by air formaldehyde" to this conference. The gene expression pattern of chemosensitive receptor VR1 in the brain stem of Sprague–Dawley neonatal rats (5 in both test and control group) was determined after low-level gaseous formaldehyde exposure (1.8mg/m^3 for 72h). The mRNA levels of VR1 and cytokine NGF gene were measured

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with RT–PCR. They suggested that formaldehyde exposure induced cytokine NGF expression and then NGF up-regulates VR1 gene expression synchronously.

3 A Japanese research group (Fujimaki H et al, 2004) published their paper "inhalation of low-level formaldehyde enhances nerver growth factor production in the hippocampus of mice" in ***Neuroimmunomodulation*** 11:373-375. They concluded that exposure of immunized mice to low levels of formaldehyde increases NGF levels in the hippocampus.

FORMALDEHYDE-INDUCED ASTHMA

This is an old topic, but recent researches are involved in new mechanisms.

1 Many experts thought that formaldehyde-induced asthma was the same asthma that mediated by "formaldehyde-specific IgE antibodies". Moreover, they thought the detection of formaldehyde-specific IgE of patient was one and only accurate evidence for the diagnosis of formaldehyde-induced asthma. For example Grammer LC et al (1993) said "Immunologically mediated asthma caused by formaldehyde is extremely rare, if it exists at all".

2 Some scientists have understood that formaldehyde induces allergical asthma might be caused not only by "formaldehyde-specific IgE antibodies" (rare), but also by some unknown mechanisms (often). Sakamoto T et al (1999) speculated formaldehyde here took effect as a "modulator" of general IgE antibody production; Carter BG (2004) wrote formaldehyde-induced asthma as "second type of allergic reaction" or "T cell allergic reaction".

3 Zhisong Lu et al (2005) have done some academic analyses and animal studies to search the unknown mechanisms. In the paper titled "Type II vanilloid receptor signaling system: one of the possible mechanisms for the rise in asthma cases" they found a reasonable explanation formaldehyde-induced asthma.

AIRWAY NEUROGENIC INFLAMMATION

Neurogenic inflammation is an important pathological mechanism, which is involved in process of formaldehyde induced airway irritation, SBS and asthma.

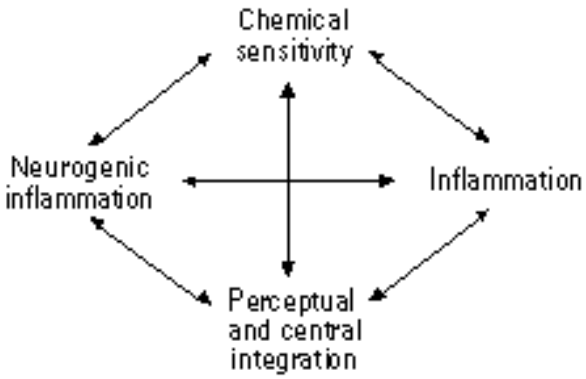

Figure 1. *Potential interactions between chemical sensitivity and the domains of neurogenic inflammation, perceptual and central integration, and nonneurogenic inflammation (Bascom R et al, 1997).*

1 Definition: Neurogenic inflammation is initiated by stimulation of peripheral c-fiber neurons (Lundberg JM et al, 1988). A peripheral axon reflex results in the release of neuropeptides (substance P, CGRP, NKA and so on) and in signs of inflammation at a peripheral sites distinct from the site of the original stimulus. The stimulus is also transmitted centrally and provides a central afferent signal and efferent reflexes.

2 In 1997, a working group recommended that studies be initiated in these areas in the article "neurogenic inflammation: with additional discussion of central and perceptual integration of nonneurogenic inflammation" (Bascom R et al, 1997).

3 He Hujun et al (2005) have carried out a human controlled exposure experiments on the relationship between air formaldehyde and neurogenic inflammation. The authors reported the content of substance P in nasal lavage increased after exposure of 3mg/m^3 formaldehyde ($p<0.05$). This maybe explained that formaldehyde could

stimulate trigeminal nerve ends of the eyes, which would release substance P from its nasal branches through axon reflex.

WORK AS SMALL GASEOUS SIGNALING MOLECULE

This hypothesis was proposed by of young members of our group.

1 Since the 1980s, biologists came to notice that some small gaseous molecules maybe act as signaling molecules. Three gaseous molecules have been discovered at present time (Zhao W et al, 2001), known as nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H_2S). Boehning D et al (2003) in their paper "Novel neural modulators" (Annu. Res. Neurosci, 26:105~131) summarized the characteristics of the three signaling molecules. These characteristics may be served as principles for novel signaling molecules.

2 The young members of our group compared and analyzed literature about signaling molecules, and hypothesized that formaldehyde may be another novel signaling molecule. The review "a new hypothesis of endogenoue formaldehyde as a novel signaling molecule" (Yi Cao et al, 2005, submitted to Indoor Air' 2005) gives their hypothesis for further research in detail.

EPIGENESIS: METHYLATION OF DNA AND PROTEINS

Methylation of DNA and histone is a main form of epigenesis. Endogenoue formaldehyde, especially "active formaldehyde", has been regarded to participate in this process.

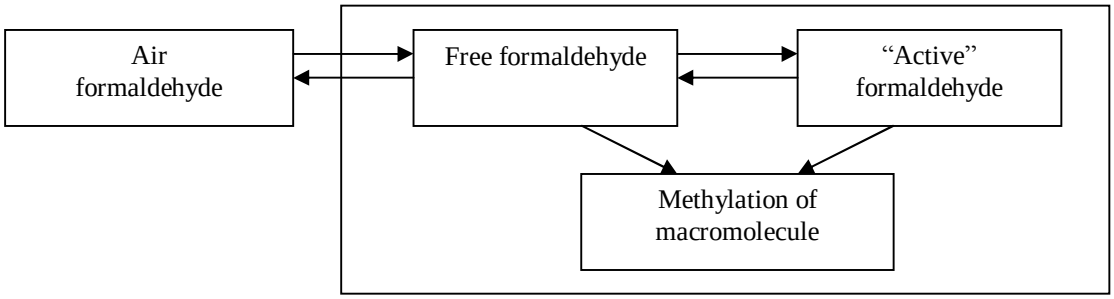

Figure 2. *The relationship between methylation of macromolecule and air formaldehyde, endogenous formaldehyde and active formaldehyde.*

1 Endogenoue formaldehyde participates in the methylation of macromolecules in two forms: free formaldehyde and "active" formaldehyde. In the cell, toxic free formaldehyde is not produced and enzymes (SDH and DMGDH) catalyse the formation of 5,10-methylene-THF (i.e. 'active' formaldehyde) from tetrahydrofolate (THF) and the labile iminium intermediate. In the absence of THF, free formaldehyde is the second product of catalysis, formed by hydrolysis of a labile iminium intermediate. (Leys D et al, 2003)

2 The simplest type of methylation occurs without any enzymatic catalysis. This procedure is called either prebiotic methylation or chemical methylation. Waddell et al. (2000) described their observation that certain prebiotic compounds (such as ethanolamine and glycine) can be methylated by an excess of formaldehyde alone.

3 Many studies have been undertaken on formaldehyde-related methylation. Kalasz H (2003) summarized these studies in his paper "biological role of formaldehyde, and cycles related to methylation, demethylation, and formaldehyde production". He indicated the formaldehyde-related methylation and demethylation of DNA may have an important role in pathogenesis of certain diseases. Lower formaldehyde may essentially cause cell survival.

4 Histones are DNA-binding proteins with relatively small molecular size. Histones are of basic character, rich in lysine and arginin, capable for N-methylation. H3 and H4 Histones of K562 erythroleukemia cells were successfully methylated by L-[methyl-3H]methionine, in the presence of cycloheximide (Beneker J et al, 1991).

DNA-PROTEIN CROSSLINK

The induction of DNA-protein crosslink has been proposed to be an important biological molecular indicator and mechanism of the carcinogenicity of formaldehyde (WHO 2002).

1 In 1996 the expert panel of American health foundation reported that (Conaway CC et al 1996): In the genetic toxicity and carcinogenic process of the gas formaldehyde, the most important part is the forming of DNA-protein crosslinks, DPC.

2 Shaham J et al (2003) published a paper titled "DNA–protein crosslinks and p53 protein expression in relation to occupational exposure to formaldehyde". The authors concluded that there were a possible causal relation between DPC and mutation in p53, and DPC could be applied to assessment of the development of cancer due to formaldehyde exposure.

3 Yingshuai Liu et al (2005) carried out a work on the genotoxicity of formaldehyde. In the paper named "Study on formation and repair of DNA damages induced by formaldehyde in vivo" (Submitted to the journal *Front. Biosci*), the authors used comet assay and SDS-KCl methods to confirm that formaldehyde could increase the level of DPC in human lymphocyes and Hela cell line at the concentrations higher than 50μM.

LYSIN-DPC REPAIR ENZYMEL

Despite the importance of DPC in carcinogenicity of formaldehyde, little is known about the repair mechanism of it.

1 Quievryn G & Zhitkovich A (2000) conducted a study on the stability of formaldehyde induced DPC in vitro and in human cells. In the paper titled "loss of DNA-protein crosslinks from formaldehyde-exposed cells occurs through spontaneous hydrolysis and an active repair process linked to proteosome", the authors suggested that cells also possess an active repair process for DPC removal in addition to spontaneous hydrolysis.

2 In 2002 Takashima et al published a paper in *nature genetics* (2002). The first DPC repair enzyme named TDP1 was reported in this paper. While TDP1 can only repair the DPC in protein-Tyr-DNA type, the repair enzyme for formaldehyde induced-DPC occurred between lysines and bases is still unknown.

3 Our group has done some work to search the specific DPC repair enzyme for formaldehyde induced-DPC. We have found that formaldehyde induced-DPC can be removed passing 16 hours in HepG 2 cell line. 2-D electrophoresis and mRNA differential display are now used to find the different proteins expressed by formaldehyde stress cells. Specific DPC repair enzyme maybe include in these proteins (Data unpublished). The "Tan Chin Tuan Exchange Fellowship" from Nanyang Technological University of Singapore funds this work.

FORMALDEHYDE-RRELATED APOPTOSIS

The relation between formaldehyde and apoptosis is complex, some studies reported formaldehyde can induce apoptosis; and the other studies reported formaldehyde can prevent apoptosis. For example:

1 Teng S et al (2001) wrote the paper "the formaldehyde metabolic detoxification enzyme systems and molecular cytotoxic mechanism in isolated rat hepatocytes", in which formaldehyde was regarded to have ability open mitochondrial permeability transition pore complaex, PTPC. This may promote cell apoptosis.

2 Szende et al (2000) described the dose-dependent effect of resveratrol on the apoptosis in endothelial cell cultures. As resveratrol (i.e. 3,5,4'-trihydroxystilbene) is reactive to formaldehyde, the resveratrol effect on the endothelial cell culture is double related to its formaldehyde capturing effects. The level of formaldehyde is regulated by resveratrol, and the reaction products (of formaldehyde captured by resveratrol) promote activity on apoptosis.

CONCLUSION

Toxicological studies and risk assessment of formaldehyde has made a great progress. Indoor air formaldehyde pollution is still a serious problem in the world. In order to understand more information about toxicities of formaldehyde, we must know more about its physiological effects in body, because its physiological effects are the bases for its toxicities.

Moreover, endogenoue formaldehyde, especially active formaldehyde may involve in very basic life phenomena, for example, formaldehyde-induced gene expression, formaldehyde-induced asthma, regulating effects as a signaling molecule, formaldehyde-induced methylation of DNA and proteins, and so on. Research on endogenoue

formaldehyde may let us have a very different angle to search the answers of some certain basic life elements.

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